FEBS Letters 581 (2007) 3899–3903

Hypothesis

Do mitochondria act as "cargo boats" in the journey of GD3 to the nucleus during apoptosis?

Tina Garofalo[a,b], Antonella Tinari[c], Paola Matarrese[d], Anna Maria Giammarioli[d], Valeria Manganelli[a], Laura Ciarlo[a,d], Roberta Misasi[a], Maurizio Sorice[a,b], Walter Malorni[d,*]

[a] Department of Experimental Medicine, "Sapienza" University, Rome, Italy
[b] Laboratory of Experimental Medicine and Environmental Pathology, "Sapienza" University, Rieti, Italy
[c] Department of Technology and Health, Istituto Superiore di Sanita', Rome, Italy
[d] Department of Drug Research and Evaluation, Section of Cell Aging and Degeneration, Istituto Superiore di Sanita', viale Regina Elena 299, 00161 Rome, Italy

Received 25 June 2007; accepted 12 July 2007

Available online 20 July 2007

Edited by Vladimir Skulachev

Abstract **Plasma membrane lipid rafts have been considered as a sort of "chamber", where several subcellular activities, including CD95/Fas-mediated pro-apoptotic signaling, can take place. Recently, we demonstrated that, after CD95/Fas triggering, raft-like microdomains could be detected in mitochondrial membranes. The mitochondrion appears as a dynamic and subcompartmentalized organelle in which microdomains might act as controllers of apoptosis-associated fission that results in the release of apoptogenic factors. Here, we hypothesize that some "small" mitochondria, possibly derived from their fission process, can reach the nuclear envelope and strictly interact with this. Mitochondria could act as a signaling "device" contributing to molecular trafficking of molecules, including raft-like components, during apoptosis.**

Keywords: Lipid rafts; Apoptosis; Mitochondria; Nucleus

1. Lipid rafts

Lipid rafts were described as a sort of "dynamic chamber", where several subcellular activities, including CD95/Fas-mediated proapoptotic signaling, take place [1,2]. From the biochemical point of view, they are specifically enriched in certain lipids (sphingolipids, including gangliosides, sphingomyelin, and cholesterol), whereas other lipids (e.g., glycerophospholipids) are selectively depleted [3]. Despite their peculiarity in lipid composition, the key role of these structures in signal transduction is strictly depending on their (glyco)protein composition. Indeed, a large variety of proteins has been detected in these microdomains isolated from different cell types, including tyrosine kinase receptors [4], mono- [5] or heterotrimeric G proteins [6], Src-like tyrosine kinases [7], PKC isozymes [8] and GPI-anchored proteins [9,10].

Lipid rafts play crucial roles in several signal transduction pathways starting from plasma membrane, thus providing a mechanism for the compartmentalization of signaling components. In fact, they can "focus" receptors for interaction with their ligands and/or effectors on both sides of the plasma membrane increasing de facto their concentration, but they can also exclude other components. This can speed up the binding process during signaling and prevent inappropriate cross talk among diverse pathways [1]. This was also demonstrated for certain components of importance in apoptosis triggering [1,11,12].

2. Raft-like microdomains on mitochondria

Previous findings have identified the ganglioside GD3 as an emerging glycolipidic intermediate in apoptotic signaling that targets mitochondria in response to death signals. Indeed, Garcia-Ruiz and colleagues characterized the trafficking of GD3 to mitochondria in response to tumor necrosis factor-α (TNF-α) in rat hepatocytes [13]. They demonstrated that GD3 is normally confined to the plasma membrane, as well as in the endosomal/Golgi network. Following TNF-α treatment, GD3 undergoes a rapid intracellular redistribution from the plasma membrane to the endosomes, as detected by using early and late endosomal markers such as Rab-5 and Rab-7 [14]. The glycolipid GD3 targets to the mitochondrion probably through actin cytoskeleton vesicular trafficking and via its interaction with ezrin [15].

Recently, we demonstrated that, after CD95/Fas triggering, raft-like microdomains could be detected on mitochondrial membranes [16]. They could represent preferential sites, where some key reactions can be catalyzed, contributing to cell death execution steps. For instance, raft-like domains, enriched in gangliosides (GD3, GM3), but with a relatively low content of cholesterol, are detectable on mitochondrial membrane(s), where Bcl-family proteins (i.e. truncated Bid and Bax) are recruited. It was suggested that these microdomains could bolster mitochondrial sub-compartmentalization hijacking human T cells towards a CD95/Fas apoptotic prone phenotype [16].

However, also other subcellular organelles, such as the endoplasmic reticulum (ER) [17,18] and the Golgi complex [19], have been shown to be involved in a sort of scrambling activity

*Corresponding author. Fax: +39 06 49903691.
E-mail address: malorni@iss.it (W. Malorni).

0014-5793/$32.00
doi:10.1016/j.febslet.2007.07.020

that follows apoptosis triggering and contributes to the progression of apoptosis. The intracytoplasmic stress resulting from apoptotic triggering also leads to a directional cytoskeleton-dependent redistribution of key molecules, including lipid raft components (e.g. GD3), to mitochondria. Recruitment of plasma membrane raft components to mitochondria might contribute to intracellular apoptotic signaling. In line with this hypothesis, we have recently shown that the ganglioside GD3 could play a role as a structural mitochondrial component involved in the opening of the mitochondrial permeability transition pore. The formation of a multimolecular complex that includes VDAC-1, Bcl-2 family and fission proteins, e.g. h-Fis, has in fact been demonstrated [16]. Mitochondrial lipid raft-like microdomains may thus instruct a sort of mitochondrial ''dynamic chamber'' where specific reactions can be catalyzed, leading to cell survival or death. In particular, ganglioside GD3 could contribute to those morphogenetic changes, e.g. changes of membrane viscosity and curvature, which could in turn lead to mitochondrial fission associated with apoptosis execution [16]. In fact, the role of gangliosides in this multimolecular complex could be to facilitate the transient and local formation of inverted hexagonal structures that undergo the fission process. Hence, they could play a key role in the onset of both outer (OMD) and inner (IMD) mitochondrial membrane dissolution [20]. Mitochondrion thus appears as a sub-compartmentalized organelle, in which microdomains may act as controllers of fission-associated morphogenetic changes that result in the release of apoptogenic factors [21].

3. Mitoptosis

Mitoptosis represents a mechanism by which mitochondria undergo extensive fragmentation and subsequent caspase-independent elimination during programmed cell death. However, mechanisms underlying these processes remain unclear. It is well known that apoptotic stimuli target mitochondria for degradation by autophagy, a catabolic process that allows recycling of cytoplasmic components, including organelles, into basic components [22–24]. We recently showed [20] that in lymphoblastoid T cells, following CD95/Fas triggering, two different additional pathways for mitochondrial execution can be observed: (i) OMD and (ii) IMD. During OMD, condensation and collapse of internal cristae precedes the disruption of outer membrane, followed by cytoplasmic spreading of mitochondrial debris. On the contrary, IMD appears to be characterized by mitochondrial coalescence, rarefaction of the mitochondrial matrix and disruption of internal cristae. This finally results in organelle extrusion (mitoptosis) [25] or, alternatively, sequestration in large autophagic vacuoles (mitophagy) [20].

Thus, the final steps of the so-called execution phase of cell death cannot merely be considered as a dissolution process. Depending on the type of death process, e.g. apoptotic, autophagic or necrotic, and of the molecular cascade, a different series of organelle alterations can be involved. Indeed, according to the cell type and the noxious agent, various organelles (mainly ER and Golgi) undergo profound changes, such as swelling and membrane remodeling. Mitochondrial fate ap-

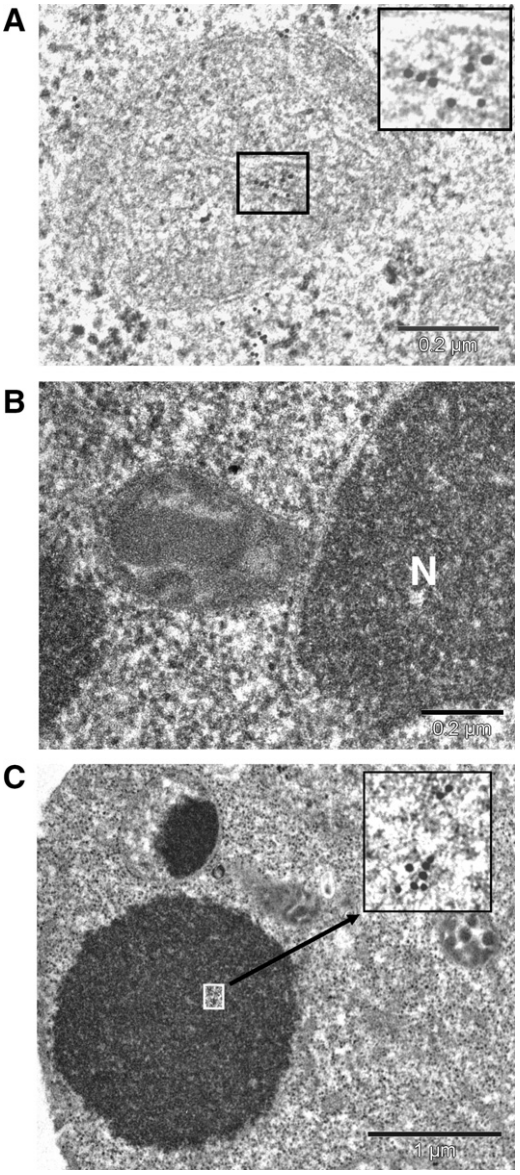

Fig. 1. Transmission electron micrographs of lymphoblastoid CEM cells undergoing apoptosis. (A) Immunogold labeling of GD3 1 h after CD95/Fas triggering. Electron micrograph shows gold particles localized at the mitochondrial cristae, Magnifications: 100000×; inset 150000. (B) High magnification electron micrograph showing a fission-derived mitochondrion in close contact with the nuclear membrane. Note the strict interaction between the electron dense small mitochondrion and the nuclear envelope in correspondence of a chromatin clump. N: nucleus. Magnification: 80000×. (C) Immunogold labeling of GD3 4 h after CD95/Fas triggering. Electron micrograph shows gold particles localized into the neo-formed micronuclei of an apoptotic cell and, in particular (inset), within the condensed and clumped chromatin. The arrow in the inset at high magnification indicates gold particles. Note the absence of gold labeling at mitochondrial level. Magnifications: 10000×; inset: 100000×. For these transmission electron microscopy analyses, CEM cells, four hours after CD95/Fas triggering, were fixed with 2.5% glutaraldehyde and 1% osmium tetroxide, dehydrated through graded series of ethanol solutions and embedded in Agar 100 resin. Immunogold labeling was performed by using anti-GD3 monoclonal antibody as primary antibody and an anti-mouse IgM-gold (10 nm) conjugate (both from Sigma, Italy). Ultrathin sections were then counterstained with uranyl acetate and lead citrate.

pears instead quite different. As a consequence of a scrambling with other organelles or with plasma membrane, including lipid rafts, molecular symmetries that instruct proper mitochondrial morphology can be deeply modified and subverted. At glance, degenerating mitochondria are generally surrounded by lysosomal membranes in order to allow the cell to re-cycle debris and molecular components for its survival. Alternatively, mitochondria dissolution can also take place in different forms, probably depending upon cell type: an IMD that is typical of cell death by necrosis (but that also occurs in the latest phases of apoptosis) and, more interestingly, an OMD, i.e. a loss of the external curvature and assembly of the outer membrane, known to be maintained among others, by glycosphingolipids.

4. "Mitochondrial invasion of the nucleus" [25]

Since 1958, it was reported the presence of mitochondria inside the nucleus [26]. However, only recent lines of evidence suggested that mitochondria could move towards the nucleus during the apoptotic process [25], although mitochondrial "invasion" of the nucleus is a rather rare case.

Apoptosis is associated with the dissolution of mitochondria filamentous network to form small rounded mitochondria, which move towards the nucleus. These mitochondria are able to release into the nucleus apoptogenic factors such as the apoptosis-inducing factor (AIF) [27], as well as endonuclease G [28,29]. Thus, as previously suggested since 2004 by Skulachev et al. [25], mitochondria might act as "cargo boats", delivering some pro-apoptotic proteins to their destination, contributing to cell death execution. It remains to be elucidated how the nuclear membrane is opened in order to allow the entrance of mitochondria into the nucleus. In this regard, a short-term opening of some area of the nuclear membrane with a consequent "healing" of the hole has been suggested [25]. Alternatively, a fusion phenomenon with nuclear membrane with release of mitochondrial content might also occur.

5. A hypothesis: from lipid rafts to cargo boats

It was suggested that "small" mitochondria might serve as a transportable form of these organelles, which, in this way, could transfer some apoptogenic factors to the nuclear target [25]. However, it has been underlined that the amount of pro-apoptotic mitochondrial proteins (i.e. cytochrome *c*) released from a single mitochondrion is too small to initiate the cell suicide cascade. The situation would be completely different if many or all mitochondria would participate to the release of these factors. In this case, the concentration could reach a critical level, sufficient to switch on the cell death program. Following this hypothesis, mitochondrial filaments are first decomposed (thread-grain transition), move towards the

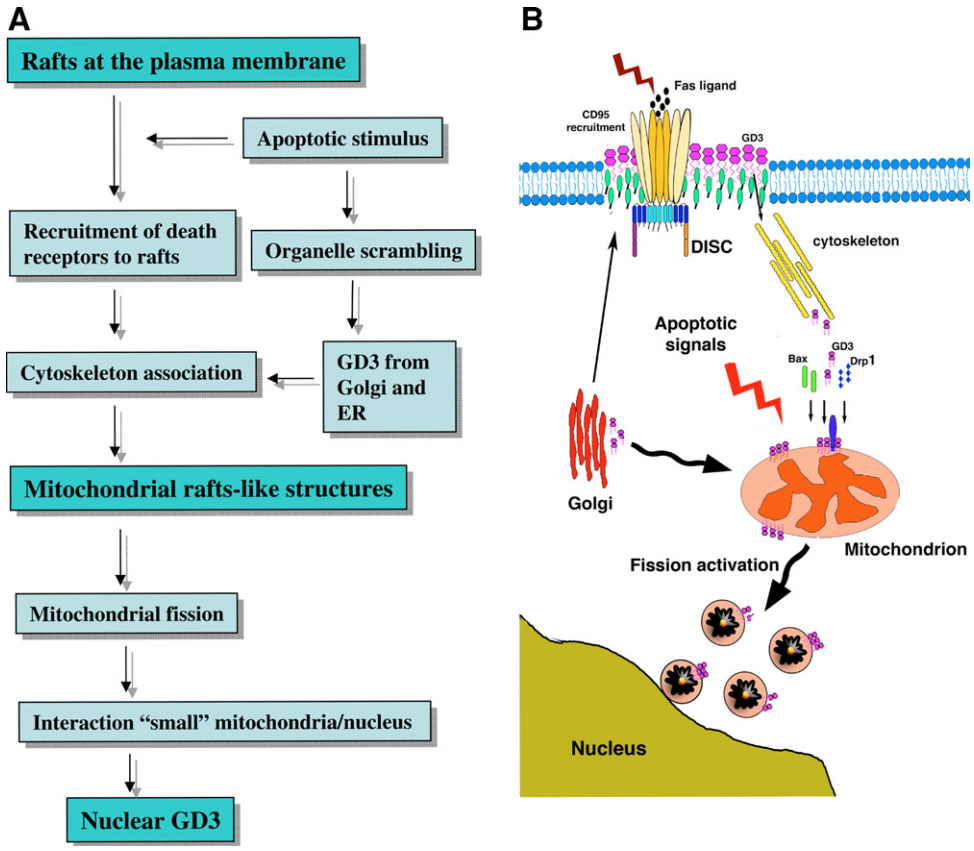

Fig. 2. (A) The suggested lipid raft "journey" inside a cell undergoing apoptosis. See text for details and references. (B) Schematic drawing illustrating the "cargo boat" hypothesis.

nucleus and release pro-apoptogenic proteins by means of the permeability transition pore-mediated swelling or, alternatively, by increasing the permeability of the outer mitochondrial membrane. It may also be the consequence of modifications of porin in the outer mitochondrial membrane, which may be carried out by Bax migrating from the cytosol to mitochondria during apoptosis [30] or by oxidation of porin by mitochondria generated super oxide anion [31].

We observed that in lymphoblastoid T cells, short time after CD95/Fas triggering (1 h), mitochondria appear to be positive for GD3, a marker of mitochondrial lipid rafts [16], as shown by gold labeling (Fig. 1A). Subsequently, small mitochondria, probably derived from fission process, can reach the nuclear envelope and strictly interact with this structure, as revealed by ultrastructural TEM analysis (Fig. 1B). Finally, at later time points after CD95/Fas triggering (4 h), immunogold labeling of GD3 was absent at mitochondrial level but, strikingly, was detectable at nuclear level (Fig. 1C). It could indicate a delivery of GD3 to the nucleus. The presence of this molecule in the nucleus has already been reported in neurons, where, during β-amyloid-induced apoptosis, it colocalizes with chromatin [32]. In addition, a colocalization of GD3 with the nucleus was also observed in neuronal cerebellar granule cells undergoing apoptosis [33]. Thus, our hypothesis is that fission-derived small mitochondria might act as signaling "devices" ("cargo boats") [25] (Fig. 2A), transporting some raft-like components, i.e. GD3, together with pro-apoptotic molecules (e.g. endoG and/or AIF), to their nuclear targets, where they may also suppress the nuclear factor-κB-dependent survival pathway [34].

Hence, the complex scenario depicted herein attempts a connection among lipid rafts, cytoskeleton, ER, Golgi apparatus and mitochondrial raft-like microdomains. In fact, ganglioside GD3 represents a lipid intermediate that targets mitochondria in response to death signals [16]. Our hypothesis (Fig. 2B) is that these organelles could act as "cargo boats" transporting GD3 into the nucleus, possibly together with other pro-apoptotic molecules, contributing to apoptosis execution.

References

[1] Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. Nature 387, 569–672.

[2] Hakomori, S., Handa, K., Iwabuchi, K., Yamamura, S. and Prinetti, A. (1998) New insights in glycosphingolipid function "signaling domain", a cell surface assembly of glycosphingolipids with signal transducer molecules, involved in cell adhesion coupled with signaling. Glycobiology 8, XI–XVIII.

[3] Brown, D.A. and Rose, J.K. (1992) Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell 68, 533–544.

[4] Pike, L., Han, X. and Gross, R.W. (2005) Epidermal growth factor receptors are localized to lipid rafts that contain a balance of inner and outer leaflet lipids. J. Biol. Chem. 280, 26796–26804.

[5] Xavier, R., Brennan, T., Li, Q., McCormack, C. and Seed, B. (1998) Membrane compartmentation is required for efficient T cell activation. Immunity 8, 723–732.

[6] Chun, M., Liyanage, U.K., Lisanti, M.P. and Lodish, H.F. (1994) Signal transduction of a G-protein-coupled receptor in caveolae: colocalization of endothelin and its receptor with caveolin. Proc. Natl. Acad. Sci. USA 91, 11728–11732.

[7] Parolini, I., Sargiacomo, M., Lisanti, M.P. and Peschle, C. (1996) Signal transduction and glycophosphatidylinositol-linked proteins (lyn,lck,CD4,CD45,G-proteins, and CD55) selectively localize in Triton-insoluble plasma membrane domains of human leukemic cell lines and normal granulocytes. Blood 87, 3783–3794.

[8] Parolini, I., Topa, S., Sorice, M., Pace, A., Ceddia, P., Montesoro, E., Pavan, A., Lisanti, M.P., Peschle, C. and Sargiacomo, M. (1999) Phorbol ester-induced disruption of the CD4-lck complex occur within a detergent-resistant microdomain of the plasma membrane. Involvement of the translocation of activated protein Kinase C isoform. J. Biol. Chem. 274, 14176–14187.

[9] Cinek, T. and Horejsi, V.J. (1992) The nature of large non covalent complexes containing glycosyl-phosphatidylinositol-anchored membrane glycoproteins and protein tyrosine kinases. Immunology 149, 2262–2270.

[10] Horejsi, V., Drbal, K., Cebecauer, M., Cerny, J., Brdicka, T., Angelisova, P. and Stockinger, H. (1999) GPI-microdomains: a role in signaling via immunoreceptors. Immunol. Today 20, 356–361.

[11] Pizzo, P., Giurisato, E., Bigsten, A., Tassi, M., Tavano, R., Shaw, A. and Viola, A. (2004) Physiological T cell activation starts and propagates in lipid rafts. Immunol. Lett. 91, 3–9.

[12] Pizzo, P. and Viola, A. (2004) Lipid rafts in lymphocyte activation. Microbes Infect. 6, 686–692.

[13] Garcia-Ruiz, C., Colell, A., Morales, A., Calva, M., Enrich, C. and Fernandez-Checa, J.C. (2002) Trafficking of ganglioside GD3 to mitochondria by tumor necrosis factor-alpha. J. Biol. Chem. 277, 36443–36448.

[14] Zerial, M. and McBride, H. (2001) Rab proteins as membrane organizers. Nat. Rev. Mol.Cell. Biol. 2, 107–117.

[15] Giammarioli, A.M., Garofalo, T., Sorice, M., Misasi, R., Gambardella, L., Gradini, R., Fais, S., Pavan, A. and Malorni, W. (2001) GD3 glycosphingolipid contributes to Fas-mediated apoptosis via association with ezrin cytoskeletal protein. FEBS Lett. 506, 45–50.

[16] Garofalo, T., Giammarioli, A.M., Misasi, R., Tinari, A., Manganelli, V., Gambardella, L., Pavan, A., Malori, W. and Sorice, M. (2005) Lipid microdomains contribute to apoptosis-associated modifications of mitochondria in T cells. Cell Death Diff. 12, 1378–1389.

[17] DiSano, F., Ferraro, E., Tufi, R., Achsel, T., Piacentini, M. and Cecconi, F. (2006) Endoplasmic reticulum stress induces apoptosis by an apoptosome-dependent but caspase 12-independent mechanism. J. Biol. Chem. 281, 2693–2700.

[18] Goetz, J.G. and Nabi, I.R. (2006) Interaction of the smooth endoplasmic reticulum and mitochondria. Biochem. Soc. Trans. 34, 370–373.

[19] Ouasti, S., Matarrese, P., Paddon, R., Khosravi-Far, R., Sorice, M., Tinari, A., Malorni, W. and Degli Esposti, M. (2007) Death receptor ligation triggers membrane scrambling between Golgi and mitochondria. Cell Death Diff. 14, 453–461.

[20] Tinari, A., Garofalo, T., Sorice, M., Degli Esposti, M. and Malorni, W. (2007) Mitoptosis: different pathways for mitochondrial execution. Autophagy 3, 282–284.

[21] Estaquier, J. and Arnoult, D. (2007) Inhibiting Drp1-mediated mitochondrial fission selectively prevents the release of cytochrome *c* during apoptosis. Cell Death Differ. 14, 1086–1094.

[22] Arnoult, D., Rismanchi, N., Grodet, A., Roberts, R.G., Seeburg, D.P., Estaquier, J., Sheng, M. and Blackstone, C. (2005) Bax/Bak-dependent release of DDP/TIMM8a promotes Drp1-mediated mitochondrial fission and mitoptosis during programmed cell death. Curr. Biol. 15, 2112–2118.

[23] Karbowsky, M. and Youle, R.J. (2003) Dynamics of mitochondrial morphology in healthy cells and during apoptosis. Cell Death Diff. 10, 870–880.

[24] Kundu, M. and Thompson, C.B. (2005) Macroautophagy versus mitochondrial autophagy: a question of fate? Cell Death Differ. 12, 1484–1489.

[25] Skulachev, V.P., Bakeeva, L.E., Chernyak, B.V., Domnina, L.V., Minin, A.A., Pletjushkina, O.Y., Saprunova, V.B., Skulachev, I.V., Tsyplenkova, V.G., Vasiliev, J.M., Yaguzhinsky, L.S. and Zorov, D.B. (2004) Thread-grain transition of mitochondrial reticulum as a step of mitoptosis and apoptosis. Mol. Cell Biochem. (256/257), 341–358.

[26] Hoffman, H. and Grigg, G.W. (1958) An electron microscopic study of mitochondria formation. Exp. Cell Res. 15, 118–131.

[27] Joza, N., Susin, S.A., Daugas, E., Standford, W.L., Cho, S.K., Li, C.Y., Sasaki, T., Elia, A.J., Cheng, H.-Y., Ravagnan, L., Ferri, K.F., Zamzami, N., Wakeham, A., Hakem, R., Yoshida, H., Kong, Y.Y., Mak, T.W., Zuniga-Pflucker, J.C., Kroemer, G. and

Penninger, J.C. (2001) Essential role of mitochondrial apoptosis-inducing factor in programmed cell death. Nature 410, 549–554.
[28] Li, L., Luo, X. and Wang, X. (2001) Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 412, 95–99.
[29] Matarrese, P., Tinari, A., Mormone, E., Bianco, G.A., Toscano, M.A., Ascione, B., Rabinovich, G.A. and Malorni, W. (2005) Galectin-1 sensitizes resting human T lymphocytes to Fas (CD95)-mediated cell death via mitochondrial hyperpolarization, budding, and fission. J. Biol. Chem. 280, 6969–6985.
[30] Shimizu, S., Narita, M. and Tsujimoto, Y. (1999) Bcl-2 family proteins regulate the release of apoptogenic cytocrome *c* by the mitochondrial channel VDAC. Nature 399, 483–487.
[31] Madesh, M. and Hajnoczky, G. (2001) VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytocrome *c* release. J. Cell Biol. 155, 1003–1015.
[32] Copani, A., Melchiorri, D., Caricatole, A., Martini, F., Sale, P., Carnevale, R., Gradini, R., Sortino, M.A., Lenti, L., De Maria, R. and Nicoletti, F. (2002) Beta-amyloid induced synthesis of the gangliosides GD3 is a requisite for cell cycle reactivation and apoptosis in neurons. J. Neurosci. 22, 3963–3968.
[33] Melchiorri, D., Martini, F., Lococo, E., Gradini, R., Barletta, E., De Maria, R., Caricasole, A., Nicoletti, F. and Lenti, L. (2002) An early increase in the disialoganglioside GD3 contributes to the development of neuronal apoptosis in culture. Cell Death Differ. 9, 609–615.
[34] Colell, A., Garcia-Ruiz, C., Roman, J., Ballesta, A. and Fernandez-Checa, J.C. (2001) Ganglioside GD3 enhances apoptosis by suppressing the nuclear factor-kB-dependent survival pathway. FASEB J. 15, 1068–1070.